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(71) Applicant *(for all designated States except US)*: THE AMERICAN NATIONAL RED CROSS [US/US]; 430 17th Street, N.W., Washington, DC 20006 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MACPHEE, Martin, J. [CA/US]; 9971 Lake Landing Road, Gaithersburg, MD 20886 (US). DROHAN, William, N. [US/US]; 8417 Oakford Drive, Springfield, VA 22156 (US). BEALL, Dawson [US/US]; 12539 Great Park Circle #101, Germantown, MD 20876 (US). FRIEDMAN, Stanley, A. [US/US]; 4317 Regalwood Terrace, Burtonsville, MD 20866 (US). TUTHILL, David [US/US]; 510 Elm Street, Frederick, MD 21701 (US). BAYER, Valdislav [-/US]; 36 Pontiac Way, Gaithersburg, MD 20878 (US).

(74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).

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(57) Abstract

The present invention relates to a haemostatic multilayer bandage that comprises preferably a thrombin layer between two fibrinogen layers. The dressing may contain other resorbable materials such as glycolic acid or lactic acid based polymers or copolymers. The inventive haemostatic sandwich bandage is useful for the treatment of wounded tissue.

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HEMOSTATIC SANDWICH BANDAGE

I. FIELD OF THE INVENTION

The present invention relates to a hemostatic sandwich bandage which comprises a plurality of layers that contain resorbable materials and/or coagulation proteins. The inventive hemostatic sandwich bandage is useful for the treatment of wounded tissue.

II. BACKGROUND OF THE INVENTION

The control of hemorrhage (bleeding) is a critical step in first aid and field trauma care. Unfortunately, the materials and methods available to stop bleeding in prehospital care (gauze dressings, direct pressure, and tourniquets) have not changed greatly in the past 2000 years. L. Zimmerman *et al.*, *Great Ideas in the History of Surgery* (San Francisco, Calif: Norman Publishing; 1993), 31. Even in good hands they are not uniformly effective, and the occurrence of excessive bleeding or fatal hemorrhage from an accessible site is not uncommon. J.M. Rocko *et al.*, *J. Trauma 22*:635 (1982).

Mortality data from Vietnam indicates that 10% of combat deaths were due to uncontrolled extremity hemorrhage. *SAS/STAT Users Guide*, 4th ed. (Cary, NC: SAS Institute Inc; 1990). Up to one third of the deaths from exsanguination during the Vietnam War could have been prevented by the use of effective field hemorrhage control methods. *SAS/STAT Users Guide*, 4th ed. (Cary, NC: SAS Institute Inc; 1990).

Although civilian trauma mortality statistics do not provide exact numbers for prehospital deaths from extremity hemorrhage, case and anecdotal reports indicate similar occurrences. J.M. Rocko *et al.*, *J. Trauma 22*:635 (1982). These data suggest that a substantial increase in survival can be effected by the prehospital use of a simple and effective method of hemorrhage control.

Liquid fibrin sealants have been used for years as an operating room adjunct for hemorrhage control. J.L. Garza *et al.*, *J. Trauma 30*:512-513 (1990); H.B. Kram *et al.*, *J. Trauma 30*:97-101 (1990); M.G. Ochsner *et al.*, *J. Trauma 30*:884-887 (1990); T.L. Matthew *et al.*, *Ann. Thorac. Surg. 50*:40-44 (1990); H. Jakob *et al.*, *J. Vasc. Surg.*, *1*:171-180 (1984). The first mention of tissue glue used for hemostasis dates back to 1909. *Current Trends in Surgical Tissue Adhesives: Proceedings of the First International Symposium on Surgical Adhesives*, M.J. MacPhee *et al.*, eds. (Lancaster, Pa: Technomic Publishing Co; 1995). The widespread use of fibrinogen and thrombin was common in the last year of World War II, but was abandoned because of the transmission of hepatitis. D.B. Kendrick, *Blood Program in WW II* (Washington, DC: Office of the Surgeon General, Department of Army; 1989), 363-368.

Currently, single donor fibrin sealants are widely used clinically, not only for hemorrhage control but in various surgical situations. W.D. Spotnitz, *Thromb. Haemost. 74*:482-485 (1995); R. Lerner *et al.*, *J. Surg. Res. 48*:165-181 (1990). Even more extensive use is limited by the strict requirements for temperature control, availability of thawed blood components, and the need for mixing of components. Additional problems with the standard fibrin sealants stem from the transfusion risk of human cryoprecipitate (E.M. Soland *et al.*, *JAMA 274*:1368-1373 (1995)), the low and variable amounts of fibrinogen in the cryoprecipitate (10-30 mg) (P.M. Ness *et al.*, *JAMA 241*:1690-1691 (1979)), hypotensive responses to bovine thrombin (R. Berguer *et al.*, *J. Trauma 31*:408-411 (1991)) and antibody responses to bovine thrombin (S.J. Rapaport *et al.*, *Am. J. Clin. Pathol. 97*:84-91 (1992)).

The American Red Cross and others have developed plasma protein purification methods that seem to eliminate the hepatitis risk. R.F. Reiss *et al.*, *Trans. Med. Rev. 10*:85-92 (1996). These products are presently being considered for approval by the Food and Drug Administration.

A dry fibrinogen-thrombin dressing (TACHOCOMB, Hafslund Nycomed Pharma, Linz, Austria) is also available for operating room use in many European countries. U. Schiele *et al.*, *Clin. Materials* 9:169-177 (1992). Present formulations of this dressing use bovine thrombin. While this fibrinogen-thrombin dressing requires no premixing and is easy to use, its utility for field applications is limited by a requirement for storage at 4°C and the necessity for prewetting with saline solution prior to application to the wound.

III. SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a hemostatic sandwich bandage that can be used for wound healing. Other objects, features and advantages of the present invention will be set forth in the detailed description of preferred embodiments that follows, and in part will be apparent from the description or may be learned by practice of the invention. These objects and advantages of the invention will be realized and attained by the compositions and methods particularly pointed out in the written description and claims hereof.

In accordance with these and other objects, a first embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded tissue in a patient which comprises: (i) a first fibrinogen layer; (ii) a thrombin layer adjacent to the first fibrinogen layer; and (iii) a second fibrinogen layer adjacent to the thrombin layer.

A second embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded tissue in a patient which comprises: (i) a resorbable material layer; (ii) a first fibrinogen layer adjacent to the resorbable material layer; (iii) a thrombin layer adjacent to the first fibrinogen layer; and (iv) a second fibrinogen layer adjacent to the thrombin layer.

A third embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded tissue in a patient which comprises: (i) a first fibrinogen layer; (ii) a resorbable material layer adjacent to the first fibrinogen layer; (iii) a thrombin layer adjacent to the resorbable material layer; and (iv) a second fibrinogen layer adjacent to the thrombin layer.

A fourth embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded in a patient which comprises: (i) a resorbable material layer; and (ii) a thrombin layer adjacent to the resorbable material layer. The resorbable material layer may also optionally contain fibrinogen.

A fifth embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded in a patient which comprises: (i) a first resorbable material layer; (ii) a second resorbable material layer adjacent to the first resorbable material layer; and (iii) a thrombin layer adjacent to the second resorbable material layer. The resorbable material layers may also optionally contain fibrinogen.

Each layer of the inventive hemostatic bandages may also optionally contain one or more suitable fillers, binding agents and/or solubilizing agents. In addition, each of the inventive hemostatic bandages may also optionally further comprise a release layer which contains a release agent and/or a backing material.

A sixth embodiment of the present invention is directed to methods for treating wounded tissue in a patient, which comprises applying any of the inventive hemostatic sandwich bandages to the wounded tissue.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed.

IV. DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications mentioned herein are incorporated by reference.

"Patient" as used herein refers to human or animal individuals in need of medical care and/or treatment.

"Wound" as used herein refers to any damage to any tissue of a patient that results in the loss of blood from the circulatory system. The tissue may be an internal tissue, such as an organ or blood vessel, or an external tissue, such as the skin. The loss of blood may be internal, such as from a ruptured organ, or external, such as from a laceration. A wound may be in a soft tissue, such as an organ, or in hard tissue, such as bone. The damage may have been caused by any agent or source, including traumatic injury, infection or surgical intervention.

"Resorbable material" as used herein refers to a material that is broken down spontaneously and/or by the mammalian body into components which are consumed or eliminated in such a manner as not to interfere significantly with wound healing and/or tissue regeneration, and without causing any significant metabolic disturbance.

"Stability" as used herein refers to the retention of those characteristics of a material that determine activity and/or function.

"Binding agent" as used herein refers to a compound or mixture of compounds that improves the adherence of one layer of the inventive hemostatic sandwich bandage to one or more different layers and/or the adherence of the components of a given layer to other components of that layer.

"Solubilizing agent" as used herein refers to a compound or mixture of compounds that improves the dissolution of a protein or proteins in aqueous solvent.

"Filler" as used herein refers to a compound or mixture of compounds that provide bulk and/or porosity to one or more layers of the inventive hemostatic sandwich bandages.

"Release agent" as used herein refers to a compound or mixture of compounds that facilitates removal of an inventive hemostatic sandwich bandage from a manufacturing mold.

"Foaming agent" as used herein refers to a compound or mixture of compounds that produces gas when hydrated under suitable conditions.

B. PREFERRED EMBODIMENTS

A first preferred embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded tissue in a patient which comprises:(i) a first fibrinogen layer; (ii) a thrombin layer adjacent to the first fibrinogen layer; and (iii) a second fibrinogen layer adjacent to the thrombin layer.

A second embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded tissue in a patient which comprises: (i) a resorbable material layer; (ii) a first fibrinogen layer adjacent to the resorbable material layer; (iii) a thrombin layer adjacent to the first fibrinogen layer; and (iv) a second fibrinogen layer adjacent to the thrombin layer.

A third embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded tissue in a patient which comprises: (i) a first fibrinogen layer; (ii) a resorbable material layer adjacent to the first fibrinogen layer; (iii) a thrombin layer adjacent to the resorbable material layer; and (iv) a second fibrinogen layer adjacent to the thrombin layer.

A fourth embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded in a patient which comprises: (i) a resorbable material layer; and (ii) a thrombin layer adjacent to the resorbable material layer. The resorbable material layer may also optionally contain fibrinogen.

A fifth embodiment of the present invention is directed to a hemostatic sandwich bandage for treating wounded in a patient which comprises: (i) a first resorbable material layer; (ii) a second resorbable material layer adjacent to the first resorbable material layer; and (iii) a thrombin layer adjacent to the second resorbable material layer. The resorbable material layers may also optionally contain fibrinogen.

Each layer of the inventive hemostatic sandwich bandages may also optionally contain one or more suitable fillers, such as sucrose.

Each layer of the inventive hemostatic sandwich bandages may also optionally contain one or more suitable binding agents, such as sucrose.

Each layer of the inventive hemostatic sandwich bandages may also optionally contain one or more suitable solubilizing agents, such as sucrose.

Each layer of the inventive hemostatic sandwich bandages may also optionally contain one or more suitable foaming agents, such as a mixture of citric acid and sodium bicarbonate.

Each of the inventive hemostatic sandwich bandages may also optionally further comprise a release layer which contains a release agent. A preferred release agent is sucrose.

Each of the inventive hemostatic sandwich bandages may also further comprise a backing material on the side of the bandage opposite the wound-facing side. The backing material may be affixed with a physiologically-acceptable adhesive or may be self-adhering (*e.g.* by having a sufficient surface static charge). The backing material may be a resorbable material or a non-

resorbable material, such as a silicone patch or plastic. Preferably, the backing material is a resorbable material.

The fibrinogen employed in the inventive hemostatic sandwich bandage is preferably Topical Fibrinogen Complex (TFC), but any suitable fibrinogen, or derivative or metabolite thereof (such as fibrinopeptide A and fibrinopeptide B), may be employed as desired. A specific fibrinogen (or fibrinogen-containing composition) for a particular application may be selected empirically by one skilled in the art. The fibrinogen may also contain Factor XIII.

TFC is a mixture of human plasma proteins which have been purified to an appropriate level and virally inactivated. A preferred aqueous solution of TFC contains 100-130 mg/mL total protein, of which at least 80% is fibrinogen. Other constituents of TFC include albumin (generally about 5-25 mg/mL); plasminogen (generally about 5 mg/mL); Factor XIII (generally about 10-40 Units/mL); and polysorbate 80 (no more than 3%). The pH of TFC is generally in the range of 7.1-7.5. Suitable TFC may also contain fibronectin.

The thrombin employed in the inventive hemostatic bandage is preferably a lyophilized mixture of human plasma proteins which have been purified to an appropriate level and virally inactivated. A preferred aqueous solution of thrombin contains thrombin at a potency of about 300 ± 50 International Units/mL. Other constituents include albumin (generally about 5 mg/mL) and glycine (generally about 0.3 M ± 0.05M). The pH of the preferred thrombin is generally in the range of 6.5-7.1.

Additionally, in each of the embodiments of the present invention, thrombin may be replaced by any of the agents known by those skilled in the art to be activators of fibrin formation. Illustrative examples of such agents are snake venoms. A specific activator of fibrin formation for a particular application may be selected empirically by one skilled in the art.

Any suitable resorbable material known to those skilled in the art may be employed in the present invention. For example, the resorbable material may be a proteinaceous substance, such as silk, fibrin, keratin, collagen and/or gelatin, or a carbohydrate substances, such as alginates, chitin, cellulose, proteoglycans (*e.g.* poly-N-acetyl glucosamine), glycolic acid polymers, lactic acid polymers, or glycolic acid/lactic acid co-polymers. Specific resorbable material(s) for a particular application may be selected empirically by those skilled in the art.

Preferably, the resorbable material is a carbohydrate substance. Illustrative examples of particularly preferred resorbable materials are sold under the tradenames VICRYL and DEXXON.

The various layers of the inventive hemostatic sandwich bandage may be affixed to one another by any suitable means known and available to those skilled in the art. Preferably, the fibrinogen layer(s) and/or the thrombin layer(s) is (are) applied as a series of quick-frozen aqueous solution layers and subsequently lyophilized or freeze-dried.

In a particularly preferred embodiment of the present invention, when the inventive bandages are prepared using a mold, a release agent, such as sucrose, is applied to the mold before the first layer of the bandage being prepared. In such embodiments, the inventive hemostatic sandwich bandage further comprises a release layer, which contains said release agent, adjacent to the (i) layer and on the opposite side from the (ii) layer.

Alternatively, a physiologically-acceptable adhesive may applied to the resorbable material and/or the backing material (when present) and the fibrinogen layer(s) and/or the thrombin layer(s) subsequently affixed thereto.

In one embodiment of the inventive sandwich bandage, the physiologically-acceptable adhesive has a shear strength and/or structure such that the resorbable material and/or backing

material can be separated from the fibrinogen layer and/or the thrombin layer after application of the bandage to wounded tissue. In another embodiment, the physiologically-acceptable adhesive has a shear strength such that the resorbable material and/or backing material cannot be separated from the fibrinogen layer and/or the thrombin layer after application of the bandage to wounded tissue.

Suitable fibrinogen and thrombin may be obtained from human or mammalian plasma by any of the purification methods known and available to those skilled in the art; from supernatants or pastes of recombinant tissue culture, viruses, yeast, bacteria, or the like that contain a gene that expresses a human or mammalian plasma protein which has been introduced according to standard recombinant DNA techniques; or from the fluids (*e.g.* blood, milk, lymph, urine or the like) of transgenic animals that contain a gene that expresses human fibrinogen and/or human thrombin which has been introduced according to standard transgenic techniques.

As a general proposition, the purity of the fibrinogen and/or the thrombin for use in the inventive hemostatic sandwich bandage will preferably be an appropriate purity known to one of ordinary skill in the relevant art to lead to the optimal efficacy and stability of the protein. Preferably, the fibrinogen and/or the thrombin has been subjected to multiple chromatographic purfication steps, such as affinity chromatography and preferably immunoaffinity chromatography, to remove substances which cause fragmentation, activation and/or degradation of the fibrinogen and/or the thrombin during manufacture, storage and/or use. Illustrative examples of such substances that are preferably removed by purification include protein contaminants, such as inter-alpha trypsin inhibitor and pre-alpha trypsin inhibitor; non-protein contaminants, such as lipids; and mixtures of protein and non-protein contaminants, such as lipoproteins.

The concentration of the fibrinogen and/or the thrombin employed in the inventive hemostatic sandwich bandage is also preferably selected to optimize both the efficacy and stability thereof, as may be determined empirically by one skilled in the relevant art. During use of the inventive hemostatic sandwich bandage, the fibrinogen and the thrombin are preferably activated at the time the bandage is applied to the wounded tissue by the endogenous fluids of the patient escaping from the hemorrhaging wound. Alternatively, in situations where fluid loss from the wounded tissue is insufficient to provide adequate hydration of the protein layers, the fibrinogen and or the thrombin may be activated by a suitable, physiologically-acceptable liquid, optionally containing any necessary co-factors and/or enzymes, prior to or during application of the hemostatic sandwich bandage to the wounded tissue.

In addition, one or more supplements may also be contained in one or more layers of the inventive hemostatic sandwich bandage, such as growth factors, drugs, polyclonal and monoclonal antibodies and other compounds. Illustrative examples of such drugs include, but are not limited to: antibiotics, such as tetracycline and ciprofloxacin, amoxicillin, and metronidazole; anticoagulants, such as activated protein C, heparin, prostacyclin (PGI_2), prostaglandins, leukotrienes, antithrombin III, ADPase, and plasminogen activator; steroids, such as dexamethasone, inhibitors of prostacyclin, prostaglandins, leukotrienes and/or kinins to inhibit inflammation; cardiovascular drugs, such as calcium channel blockers, vasodilators and vasoconstrictors; chemoattractants; local anesthetics such as bupivacaine; and antiproliferative/antitumor drugs such as 5-fluorouracil (5-FU), taxol and/or taxotere; antivirals, such as gangcyclovir, zidovudine, amantidine, vidarabine, ribaravin, trifluridine, acyclovir, dideoxyuridine and antibodies to viral components or gene products; cytokines, such as α- or β- or γ-Interferon, α- or β-tumor necrosis factor, and interleukins; colony stimulating

factors; erythropoietin; antifungals, such as diflucan, ketaconizole and nystatin; antiparasitic agents, such as pentamidine; anti-inflammatory agents, such as α-1-anti-trypsin and α-1-antichymotrypsin; anesthetics, such as bupivacaine; analgesics; antiseptics; and hormones. Other illustrative supplements include, but are not limited to: vitamins and other nutritional supplements; glycoproteins; fibronectin; peptides and proteins; carbohydrates (both simple and/or complex); proteoglycans; antiangiogenins; antigens; lipids or liposomes; oligonucleotides (sense and/or antisense DNA and/or RNA); and gene therapy reagents.

The following examples are illustrative only and are not intended to limit the scope of the invention as defined by the appended claims. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods of the present invention without departing from the spirit and scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

All patents and publications referred to herein are expressly incorporated by reference.

V. EXAMPLES

A. EXAMPLE I

Fibrinogen and thrombin vials were removed from refrigerator and allowed to warm to room temperature for 2 hours. To each vial of thrombin 2mL of 40mM $CaCl_2$ was added, this yielded a final concentration of 500U/mL of thrombin. Dispo molds (Baxter) size 3.0 X 2.4 cm were placed on freezing tray on top of dry ice.

To each of 12 molds, 1.75 mL of H_2O was added and allowed to freeze for 1 hour at -80°C. Once frozen 140μL of thrombin (70 units) was pipetted on top of the H_2O and allowed to

freeze for an additional 1 hour at -80°C. Fibrinogen was solubilized with water (8mL) to a concentration of 50mg/mL. To the molds 1.1 mL of fibrinogen was added (55mg) and allowed to freeze for an additional 1 hour at -80°C, followed by the addition of 1mL of H_2O. The bandages were allowed to freeze for 1 hour at -80°C. Then 0.25ml of thrombin (125 units) was added and allowed to freeze for one hour. An additional 1.1 mL of fibrinogen (55mg) was pipetted on top and allowed to freeze for 1 hour.

Once all materials were added and frozen, VICRYL was placed on top and pressed into placed, by gentle finger pressure. To cover the VICRYL 500μL of 30% sucrose was added and allowed to freeze. The bandages were placed at -80°C for 2 hours, then placed into the freeze dryer. Upon completion, vacuum was released and the bandages were removed and examined.

RESULTS: The bandages when removed from the freeze dryer were uniform and consistent, the VICRYL was attached and all layers were intact.

B. EXAMPLE II

The fibrinogen and thrombin vials were removed from the refrigerator and allowed to warm to room temperature for 2 hours. Vicryl^{TM} was applied and pressed into Dispo plastic molds (2.4cm x 2.4cm). The molds were incubated at -80°C for 1 hour.

Fibrinogen was solubilized with 21mL of 2% sucrose or water to a concentration of 19.2mg/mL. In addition, fibrinogen was solubilized to a concentration of 1mg/mL with either 2% sucrose or water. The molds received 1.23mL of fibrinogen and then were frozen at -80°C for 1 hour.

Thrombin was solubilized with 0.5mL of 40mM $CaCl_2$ to a concentration of 2000U/mL. The bandages were removed from the freezer and placed on dry ice. Thrombin was sprayed at 37.5U/cm^2. The bandages were returned to -80°C for an additional 1 hour.

The bandages were placed on dry ice and received a second layer of fibrinogen (identical to the first layer). Half of the bandages were returned to -80°C for 2 hours and the other half of the bandages were placed at -20°C for 2 hours. Then, the bandages were placed in the freeze-dryer.

The bandages were subjectively assessed for resorbable material adherence and bandage appearance. Table 3 summarizes the experimental design.

Table 3. Various bandage configurations and formulations

Bottom Layer	Second Layer	Third Layer	Fourth Layer	Freeze temp prior to placement into freeze-dryer	Bandage group
Fibrinogen 1mg/cm^2 in 2% sucrose	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	1
Fibrinogen 1mg/cm^2 in water	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	2
Fibrinogen 8mg/cm^2 in 2% sucrose	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	3
Fibrinogen 8mg/cm^2 in water	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	4
VICRYL™	Fibrinogen 1mg/cm^2 in 2% sucrose	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	5
VICRYL™	Fibrinogen 1mg/cm^2 in water	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	6
VICRYL™	Fibrinogen 8mg/cm^2 in 2% sucrose	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	7
VICRYL™	Fibrinogen 8mg/cm^2 in water	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	-20°C or -80°C	8

RESULTS: The results from table 3 showed that the best bandage was . This was true for bandages frozen at both -20°C or -80°C.

C. EXAMPLE III

This bandage configuration had Vicryl™ and thrombin "layered" in between two layers of fibrinogen. The first layer of fibrinogen was solubilized in either 2% sucrose or water. The bandages were frozen at -20°C or -80°C prior to placement into the freeze-dryer. The experimental design is

outlined in Table 4. The bandages were subjectively observed for physical appearance after freeze-drying and handling characteristics after hydration.

Table 4. "Layered" bandage configuration

Bottom Layer	Second Layer	Third Layer	Fourth Layer	Fifth Layer	Freeze temp prior to placement into freeze-dryer	Group
2% Sucrose	Fibrinogen 1mg/cm^2 in 2% sucrose	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	- 20°C or - 80°C	1
2% Sucrose	Fibrinogen 1mg/cm^2 in water	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	- 20°C or - 80°C	2
2% Sucrose	Fibrinogen 8mg/cm^2 in 2% sucrose	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	- 20°C or - 80°C	3
2% Sucrose	Fibrinogen 8mg/cm^2 in water	VICRYL™	Thrombin in 40mM $CaCl_2$	Fibrinogen 8mg/cm^2 in 2% sucrose	- 20°C or - 80°C	4

RESULTS: Two structures for layered bandage production; group 7 at -20°C and -80°C from table 3 and group 1 at -20°C and -80°C from table 4, were exhibited the best characteristics in terms of no separation of layers and attachment of resorbable material.

D. EXAMPLE IV

Fibrinogen and thrombin were removed from the refrigerator and allowed to warm to room 25°C for 2 hours. For groups 1 + 2 each vial of fibrinogen received 15.3mL of 2% sucrose, final concentration of 26.2mg/mL; bandages for group 3 had 21mL of 2% sucrose to give a final concentration of 19.2mg/mL. The final amount of fibrinogen was 8mg/cm^2 for all three groups.

Dispo molds (3.0 X 2.4 cm) were placed on a freezing tray on top of dry ice. 1mL of 2% sucrose was added and allowed to freeze at -80°C for 1 hour, once frozen VICRYL was placed on

top and pressed into place. Groups 1 + 2 received 2.2mL of fibrinogen, whereas group 3 received 1.5mL of fibrinogen..

All bandages were allowed to freeze at -80°C for 2 hours. Group 1 was kept at -80°C until placed into the freeze dryer. Thrombin was solubilized with 0.5mL of 40mM $CaCl_2$ to a concentration of 2000U/mL. Groups 2 & 3 were sprayed with thrombin, so that each bandage received 144U/bandage while on dry ice. Once thrombin was applied the bandages were placed at -80°C for 1 hour. Group 3 was removed from the freezer and placed on dry ice, and an additional 1.5mL of fibrinogen at 19.2mg/mL was added.

When finished all bandages were returned to -80°C for another 2 hours. The bandages were then place into the freeze dryer, after 96 hours a sample of each group of bandages were removed and moisture content determined. Secondary drying was initiated for 24 hours. When the bandages were removed, moisture content was measured again.

RESULTS: As can be seen in table 5 the moisture content of the bandages decreases with secondary drying. All bandages looked identical and had the same texture.

Table 5

Bandage Group	% Moisture	
	Pre-Secondary Drying	Post-Secondary Drying
Fibrinogen alone (1)	4.2	3.8
Fibrinogen/Thrombin (2)	4.6	3.3
Fibrinogen/Thrombin/Fibrinogen (3)	4.3	3.9

E. EXAMPLE V

Fibrinogen and thrombin were removed from the refrigerator and allowed to warm to room 25°C for 2 hours. Dispo molds of size 3.0 X 2.4 cm were sprayed with 300μL of 2% sucrose on dry

ice, VICRYL was applied and pressed into place and the molds placed at -80°C for 1 hour. Fibrinogen was solubilized with 11mL of 2% sucrose, to a concentration of 36.4mg/mL. Each bandage had a final fibrinogen amount of 15mg/cm^2.

The molds received 1.5mL of fibrinogen and then were frozen at -80°C for 1 hour. Thrombin was solubilized with 0.5mL of 40mM $CaCl_2$ to a concentration of 2000U/mL. All bandages were sprayed with thrombin, while on dry ice and received 37.5U/cm^2.

The bandages were returned to -80°C for an additional 1 hour. The bandages were placed on dry ice and received a second layer of fibrinogen, identical to the first layer. The bandages were again returned to -80°C for 2 hours, until placed into the freeze dryer.

RESULTS: The bandages were removed from the freeze dryer and a sample of the 3.0 X 2.4 cm bandages were analyzed for moisture content. These bandages had a moisture content of 2.49%. The bandages were complete and had no separation of layers, and the VICRYL was well attached.

F. EXAMPLE VI

Forty-four square petri dishes with a size of 10.1 X 10.1 cm, with a surface area of 103cm^2 were placed on shelf trays and received 20mL of 2% sucrose, which is equal to 194μL/cm^2. Once the molds were filled they were placed at -80°C for 2 hours until frozen, and then VICRYL applied.

Fibrinogen and thrombin were removed from the refrigerator and allowed to warm to 25°C for 2 hours. Each vial of fibrinogen was solubilized with 10mL of 2% sucrose. The concentration of fibrinogen when reconstituted was 16mg/mL. The molds received 25mL of fibrinogen and where returned to -80°C for 2 hours until frozen. Thrombin was solubilized with 0.5mL of 40mM $CaCl_2$

to a concentration of 2000U/mL, and sprayed on top of the fibrinogen and returned to -80°C for 1 hour. An additional 25mL of fibrinogen was added and allowed to freeze at -80°C for 2 hours.

The bandages were then freeze dried, upon completion the bandages were packaged and sent for *in vivo* testing, described *supra.*

G. EXAMPLE VII

Fibrinogen and thrombin were removed from the refrigerator and allowed to warm to 25°C for 2 hours. Dispo plastic molds (3.0cm x 2.4cm) were placed on dry ice and sprayed with 300μL of 2% sucrose. Vicryl™ or calcium alginate was applied and pressed into place. The molds were incubated at -80°C for 1 hour.

The calcium alginate was used in two ways. In the first method, the calcium alginate was cut to the same size section as the Vicryl™ sections. In the second method, the calcium alginate was also cut to the same size section as the Vicryl™ sections, but then it was shredded into fine pieces of material (Table 6).

Fibrinogen was solubilized with 11mL of 2% sucrose to a concentration of 36.4mg/mL. Each bandage had a final fibrinogen amount of 15mg/cm^2. The molds received 1.55mL of fibrinogen and then were frozen at -80°C for 1 hour. Thrombin was solubilized with 0.5mL of 40mM $CaCl_2$ to a concentration of 2000U/mL. The bandages were placed on dry ice and sprayed with thrombin to yield 37.5U/cm^2.

The bandages were returned to -80°C for 2 hours until they were placed into the freeze-dryer. After the bandages were freeze-dried, they were tested in the porcine arteriotomy bandage performance test.

Table 6. Bandage resorbable material combinations using Vicryl™ and Calcium alginate

Bandage	Vicryl	Calcium Alginate
1	No	Whole
2	No	Shredded
3	Yes	Shredded
4	Yes	Whole

Porcine Arteriotomy Bandage Performance Test

Obtain frozen porcine aorta and thaw. Aortas can be thawed overnight at 4 °C, or individually wrapped in the water bath at 37°C. Dissect excess connective tissue from approximately first 11 cm of the aorta. Usually, the first 5-5.5 cm are free from collateral vessels. The next 5-5.5 cm should not have more than 1-2 collaterals. These can be easily sealed or patched with cyanoacrylate glue.

Cut the aorta into two 5.5 cm pieces. Invert aorta exposing the interior using a hemostat or blunt forceps. Wash both the interior and exterior of the vessel with 1-5 mL of PBS at 37°C. Stretch an O-ring over a 20cc syringe with an approximately 0.6 cm (0.25 in) hole drilled into one side. Using fingers or hemostats pull the vessel onto the syringe. Fit another O-ring of the same size onto the bottom.

Using curved hemostats, carefully secure both O-rings over the top of the vessel. The distance between both O-rings should be 3.5 cm. The artery should be snug fitting and held securely in place. Position the secured vessel such that the hole in the syringe lies in the middle of the distance between the O-rings.

Fill the syringe with PBS at 37°C and place the screw through the outside of the syringe and into the plunger, so that the plunger is held in a stationary position. Wash the artery on the syringe with 1-2ml of PBS at 37°C. Using a 16-gauge needle, make a hole in the center (approximately 1.75